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TECHNICAL NOTE

Allele frequencies and diversity parameters of 27 single nucleotide polymorphisms within and across goat breeds

I. CAPPUCCIO,* L. PARISET,* P. AJMONE-MARSAN,† S. DUNNER,‡ O. CORTES,‡ G. ERHARDT,§ G. LÜHKEN,§ K. GUTSCHER,§ S. JOOST,¶ I. J. NIJMAN,** J. A. LENSTRA,** P. R. ENGLAND,†† S. ZUNDEL,†† G. OBEXER-RUFF,‡‡ A. BEJA-PEREIRA,§§ A. VALENTINI* and THE ECONOGENE CONSORTIUM¶¶

*Dipartimento di Produzioni Animali, Università della Tuscia, Via San Camillo de Lellis, 01100 Viterbo, Italy, †Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29100 Piacenza, Italy, ‡Departimento de Producción Animal, Universidad Complutense, 28040 Madrid, Spain, §Institut für Tierzucht und Haustiergenetik, Justus-Liebig-Universität, D-35390 Giessen, Germany, ¶Ecole Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland, **Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands, ††Laboratoire d'Ecologie Alpine, Université Joseph Fourier, BP 53, F-38041 Grenoble Cedex 09, France, ‡‡Institute of Genetics, Veterinary Faculty, University of Berne, Bremgartenstrasse 109a, PO Box 8466, CH-3001 Berne, Switzerland, §§CIBIO-University of Porto, Campus Agrario de Vairão, 4485-661Vairão, Portugal, ¶¶http://lasig.epfl.ch/projets/econogene

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Abstract

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Single-nucleotide polymorphisms (SNPs) are useful markers for biodiversity assessment, linkage analysis, traceability and paternity testing. To date, there are no available SNPs for goat in the NCBI dbSNP database and only a few are reported in the literature. Within the European Union Econogene project, we characterized 27 SNPs in goats using a targeted-gene approach. Polymorphisms were identified in a panel of 16 unrelated individuals belonging to eight different goat breeds selected throughout Europe. Genotypes of 30 goats from each of the eight breeds were determined for all the SNPs characterized and diversity measures were estimated. The caprine SNPs described will be a useful complement to the available genome markers.

Keywords: gene diversity, goat, selection, single-nucleotide polymorphisms

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Ideal genetic markers for population and evolutionary studies should be abundant and distributed widely across the genome, whereas genotyping data must be comparable across laboratories with different scoring methods (Sunnucks 2000). These requirements are fulfilled by the single-nucleotide polymorphisms (SNPs) (Brouillette *et al*. 2000; Sachidanandam *et al*. 2001; Shubitowski *et al*. 2001), which have been shown to be informative suitable for ecological and conservation studies (Vignal *et al*. 2002; Brumfield *et al*. 2003; Morin *et al*. 2004; Seddon *et al*. 2005), for estimation of parameters such as population history and for inference of relationships (Kuhner *et al*. 2000; Glaubitz *et al*. 2003). Furthermore, SNPs are cost-effective for high-throughput and accurate linkage or association studies (Syvanen 2001; Vitalis *et al*. 2001; Vignal *et al*. 2002; Schlötterer 2004). However, in spite of these obvious advantages and their increasing use in human and model organism studies, SNPs have not been employed frequently in studies of nonmodel organisms, which is primarily due to a lack of availability.

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Within the Econogene project, we have selected 23 genes involved in key metabolic pathways or potentially relevant for production traits. Primers were designed from goat sequences available at GenBank, or from sequences of related species also available at GenBank. Initially, a panel of 16 unrelated individuals belonging to eight goat breeds from different European regions were used for SNP discovery. This procedure permitted us to avoid ascertainment bias on our heterozygosity estimations by using samples that represented a wide geographical area and

Correspondence: Lorraine Pariset, Fax: +39 761 357434; E-mail: pariset@unitus.it

environments and thus representing the majority of the genetic variation of Goat's gene pool: German Alpine (Germany), Corsican (France), Verata (Spain), Greek goat (Greece), Grigia Molisana (Italy), Baladie (Egypt), Polish fawn improved goat (Polony), Brava (Portugal). The authenticity of the amplification was checked by BLAST searches. A requirement to accept an SNP as authentic (not a sequencing error) and sufficiently polymorphic was that at least two copies of the rarer allele be observed (out of the 32 chromosomes observed).

SNPs were identified in exons, introns and 5′- or 3′-flanking regions (Table 1). In four genes, two SNPs were identified. Of the 27 SNPs, 18 were transitions, eight were transversions and one was a deletion.

The SNPs were subsequently typed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), PCR single-strand conformation polymorphism (SSCP), SnaPshot, or *Taq*Man via custom service (K-Bioscience). We genotyped 30 individuals from each of the eight breeds caring that at most only three individuals per farm were analysed. The program POWERMARKER (Liu & Muse 2001) was used to compute the frequencies of the rare allele, the expected heterozygosity (H_E), the observed heterozygosity (H_O) (Weir 1996), polymorphic information content (PIC) value (Botstein *et al*. 1980) and *F* statistics of genetic differentiation (Weir & Cockerham 1984) (Table 1), and the program GENECLASS2 (Piry *et al*. 2004) was used to provide allele frequencies, heterozygotes proportion and Nei's gene diversity for each breed (Table 2).

Expected heterozygosity values ranged from 0.549 (MEG3) to 0.051 (IL2_1) with a mean of 0.358; observed heterozygosity values from 0.475 (DES) to 0.040 (IL2_1) with a mean of 0.290. Except one of the two SNPs in the interleukin-2 gene, all SNPs have a frequency of the rare allele higher than 5% over all breeds and are suitable for genetic analysis. F_{ST} values are variable within the range of 0.004–0.224, but suggest that breed differentiation by a panel of well-selected SNPs is feasible. The assignment of individuals performed by GENECLASS2 software, using the criterion of Paetkau *et al*. (1995), assuming default allelic frequency of 0.001 and a threshold of 0.05, allocated correctly 59.1% of the individuals using all loci and 61.5% excluding MSTNG-5 and PRP_IN2 loci, less variable among populations. Assignment of groups of individuals under the same conditions using all loci resulted in 100% of corrected assignments. Biases can arise when transferring SNP markers across populations (Morin *et al*. 2004); if these markers are used to analyse non-European breeds, a potential limitation of ascertainment bias should be accounted for.

So far, only few SNPs in goats have been reported in the literature. This study may be a further step towards the exploitation of the vast potential of SNP-based typing within and across goat breeds for a variety of purposes.

Table 1 SNP genotyping and diversity parameters in goat genes

Locus	Name	Primers 5′–3′ (forward/reverse)	Genotyping method	SNP	Location	Rare allele frequency	*N*	H_E	H_O	PIC	F_{ST}
ACVR2B	Activin receptor IIB	GCCTTCTGGATGTTACCGAC/ TCCTTTCTGTCACTCTGGGC	*Taq*Man	U57707:g.824+102G>A	Exon 3	0.177	243	0.291	0.255	0.249	0.054
CAST	Calpastatin	AAAGAAGAAGGGATCGCAGG/ ATCAGAAGTGCTGCTGCTCCA	*Taq*Man	U66320:g.1019+345A>G	Intron 14	0.097	242	0.175	0.161	0.160	0.100
MEG3	Callipyge	TCCGAGCTCCAATAATCTTC/ TCCTTGACACGTAAGCATGG	SNaPshot	XM_872707:r.3218u>s	3′ UTR†	0.074	237	0.549	0.414	0.459	0.117
CSN1S1_1	αS1-casein	TTCTAAAAGTCTCAGAGGCAG/ GGGTTGATAGCCTTGTATGT	PCR-RFLP*	X56462:g.275delC	Exon 9	0.446	242	0.494	0.339	0.372	0.122
CSN1S1_2	αS1-casein	GGGAAAATACCCTGCAGAAG/ AAATGGAATGGCATTGTTCTA	PCR-RFLP	AY687344:g.793A>G	5′ UTR	0.349	235	0.454	0.281	0.351	0.224
CSN3	κ-casein	GGTATCCTAGTTATGGACTCAAT/ GTTGAAGTAACTTGGGCTGTGT	PCR-SSCP†	X60763:c.418G>A	Exon 4	0.246	238	0.371	0.349	0.302	0.019
CTSK	CathepsinK	TCTCACGGTTCTACTGCTGCC/ ACTGGCCATGAACCACTTGG	*Taq*Man	AY737515:g.180A>T	Intron 2	0.044	229	0.084	0.079	0.080	0.057
DES	Desmin	AGGAGATGATGAGTACCGCC/ CTGACCACCACCAGCACCCAT	*Taq*Man	AB011673:g.997+338G>A	Intron 5	0.400	240	0.480	0.475	0.365	0.069

Table 1 *Continued*

Locus	Name	Primers 5′–3′ (forward/reverse)	Genotyping method	SNP	Location	Rare allele frequency	*N*	H_E	H_O	PIC	F_{ST}
HLA-DQA_1	MHC class II DQA gene	GAAGCCCACAATGTTTGATAGTCA/ GGGGAAGAACAACAAAGAGAGGCA	*Taq*Man	AY464654:g.156A>T‡	Exon 2	0.214	227	0.336	0.181	0.280	0.156
HLA-DQA_2	MHC class II DQA gene	GAAGCCCACAATGTTTGATAGTCA/ GGGGAAGAACAACAAAGAGAGGCA	*Taq*Man	AY464654:g.207A>G‡	Exon 2	0.213	195	0.335	0.118	0.279	0.081
HLA-DRB	MHC class II DRB gene	GGGGCTTCTGGACAGATACTT/ CGCTGCACAGTGAAACTCTC	*Taq*Man	AB008362:g.153A>G	Exon 2	0.235	168	0.360	0.327	0.295	0.058
FABP4	Fatty acid-binding protein 4	CTACGCGAGCCTGGATGATAAGATGGTGCT/ ATACAACGGAATCAACCATAAAGAGAAAACTC	*Taq*Man	X89244:g.409+59A>G	3′ UTR	0.024	227	0.047	0.040	0.046	0.050
FN1	Fibronectin	CTGAACCCGGTAACGAAG/ CTCTGGGAATCTTCTCTGT	*Taq*Man	AY935993:g.221C>T	Intron 15	0.246	191	0.371	0.272	0.302	0.065
GDF9	Growth differentiation factor 9B	ACTCCGCTTCGTGTGTCAGC/ TACTCCCATTTGCCTCAATC	SNaPshot	AF236079:g.1145T>G	Exon 2	0.191	246	0.309	0.220	0.261	0.068
GHR	Growth hormone receptor	TATGCCCAGGTAAGCGACAT/ ATTGAGTACGAGGCCCTGTG	*Taq*Man	AY292282:g.77C>T	Exon 10	0.431	224	0.490	0.433	0.370	0.090
IL2_1	Interleukin-2	CTTCCTGTTTAATCAACAAATCT/ AACCTTGGGCATGTAGAAGT	PCR-RFLP	AF535145:g.88C>T	5′ flanking	0.026	247	0.051	0.053	0.050	0.004
IL2_2	Interleukin-2	CACCTCATCTGAGGAGAAGAA/ CCAGCCACTATCTGAGTACTT	PCR-RFLP	AF535145:g.931G>T	Intron 2	0.239	243	0.363	0.313	0.297	0.148
IL4	Interleukin-4	TCACATTGTCAGTGCAAATAGAG/ TTTGGGGCAGCAAAGACGT	SNaPshot	U14131:g.900C>T	Exon 2	0.461	246	0.497	0.386	0.374	0.152
ITGB1	Integrin B1	GTCTGCTACAGGCAGCTC/ CGGTGTAGTTAGGGTTGCACT	*Taq*Man	AY787746:g.253G>T	Intron 8	0.268	231	0.393	0.381	0.316	0.055
LGB	β-lactoglobulin	ACTCTTGTGGGGTGACCTGT/ CCTCCCTGGTTCCTGAAAGT	*Taq*Man	Z33881:g.6751A>G§	Exon 7	0.237	215	0.362	0.251	0.296	0.081
LIPE	Lipase	CGTTCCTGCAGACCATCT/ CCTCAATCTCGGTGATATTCC	*Taq*Man	AY935992:g.123A>C	Intron 3	0.366	220	0.464	0.414	0.356	0.046
MTNR1A	Melatonin	AGCTGTATAGCGGCACGAAT/ CCTGCCTGAAATTTTGGTTC	*Taq*Man	AF419334:g.421C>T¶	Exon 2	0.370	219	0.466	0.438	0.357	0.055
GDF8	Myostatin	CCCTCCCTTTACTGTCATCC/ ATCAAGCCCAAAATCTCTCC	*Taq*Man	AY032689:g.2121A>G	Exon 3	0.058	216	0.109	0.088	0.103	−0.012
PRNP_1	Prionprotein	TGTGGCCATGTGGAGTGACGT/ CTGGGCTTGTTCCACTGACTG	PCR-RFLP	X91999:g.145A>G	Exon 3	0.469	245	0.498	0.449	0.374	0.090
PRNP_2	Prionprotein	ATGATCTCAGCACCTACCTTG/ ATAAGAGGCCTGCTCATGGCA	PCR-RFLP	DQ366112:g.144C>T	Intron 2	0.465	215	0.498	0.437	0.374	0.056
TLR4	Toll-like receptor 4	TTCAAGGGTTGCTGTTCTCA/ CAGCACCTGAAGGCTAGAGAG	SNaPshot	AY297043:g.1414T>C	Exon 3	0.492	246	0.500	0.431	0.375	0.168
U80	*Capra hircus* microsatellite	AGTATCTTTTCTTGCATTTGTTTCC/ CACAGGGGTTTCTGGTTGG	SNaPshot	U80588:g.201A>C	Microsatellite	0.191	246	0.309	0.252	0.261	0.043

N, number of genotyped animals; H_E, expected heterozygosity of gene diversity; H_O, observed heterozygosity; PIC, polymorphic information content; F_{ST}, genetic differentiation parameters of breeds versus total. *Ramunno *et al*. 2000; †Prinzenberg *et al*. 2005; ‡Amills *et al*. 2005; §Pena *et al*. 2000; ¶Migaud *et al*. 2002.

Table 2 Allele frequencies, heterozygotes proportion and Nei's gene diversity (Nei 1987) for each locus and each breed

	Populations								
Loci	German alpine	Verata	Corsican	Greek goat	Grigia molisana	Baladie	Polish fawn imp. goat	Brava	Means
ACVR2Ch353_SNP2									
Frequencies A	0.774	0.933	0.8	0.717	0.919	0.677	0.817	0.95	0.823
Frequencies G	0.226	0.067	0.2	0.283	0.081	0.323	0.183	0.05	0.177
Heterozygotes proportion	0.387	0.133	0.333	0.5	0.161	0.065	0.367	0.1	0.256
CALPA_ChRev345_SNP2									
Frequencies A	0.081	0.333	0.052	0.033	0.032	0.032	0.083	0.133	0.098
Frequencies G	0.919	0.667	0.948	0.967	0.968	0.968	0.917	0.867	0.902
Heterozygotes proportion	0.161	0.4	0.103	0.067	0.065	0.065	0.167	0.267	0.162
CALSNP385R									
Frequencies C	0.435	0.29	0.274	0.306	0.677	0.341	0.4	0.233	0.37
Frequencies G	0.548	0.71	0.661	0.629	0.323	0.182	0.533	0.75	0.542
Frequencies T	0.016	0	0.065	0.065	0	0.477	0.067	0.017	0.088
Heterozygotes proportion	0.29	0.387	0.452	0.484	0.387	0.545	0.433	0.367	0.418
CSN1_EX9									
Frequencies delC	0.435	0.55	0.741	0.177	0.581	0.452	0.448	0.2	0.448
Frequencies wild	0.565	0.45	0.259	0.823	0.419	0.548	0.552	0.8	0.552
Heterozygotes proportion	0.419	0.5	0.172	0.097	0.774	0.452	0.276	0	0.336
CSN1S1_5									
Frequencies A	0.7	0.935	0.823	0.452	0.667	0.095	0.613	0.75	0.629
Frequencies G	0.3	0.065	0.177	0.548	0.333	0.905	0.387	0.25	0.371
Heterozygotes proportion	0.267	0.129	0.355	0.258	0.333	0.19	0.387	0.3	0.277
CSN3_Ex4									
Frequencies A	0.823	0.645	0.839	0.806	0.79	0.795	0.71	0.633	0.755
Frequencies G	0.177	0.355	0.161	0.194	0.21	0.205	0.29	0.367	0.245
Heterozygotes proportion	0.355	0.452	0.323	0.258	0.29	0.227	0.452	0.4	0.345
CTSK-G-2									
Frequencies A	0.065	0	0	0.037	0.052	0	0.173	0.033	0.045
Frequencies T	0.935	1	1	0.963	0.948	1	0.827	0.967	0.955
Heterozygotes proportion	0.129	0	0	0.074	0.103	0	0.269	0.067	0.08
DESMIN_Ch315_SNP1									
Frequencies A	0.468	0.467	0.267	0.45	0.268	0.177	0.55	0.55	0.4
Frequencies G	0.532	0.533	0.733	0.55	0.732	0.823	0.45	0.45	0.6
Heterozygotes proportion	0.484	0.533	0.4	0.433	0.464	0.355	0.567	0.567	0.475
DQA_Ch156_SNP1									
Frequencies A	0.967	0.466	0.759	0.897	0.946	0.893	0.672	0.685	0.786
Frequencies T	0.033	0.534	0.241	0.103	0.054	0.107	0.328	0.315	0.214
Heterozygotes proportion	0.067	0.379	0.185	0.138	0.107	0.071	0.31	0.185	0.18
DQA_Ch207_SNP2									
Frequencies A	0.111	0.238	0.06	0.431	0.24	0.288	0.024	0.262	0.207
Frequencies G	0.889	0.762	0.94	0.569	0.76	0.712	0.976	0.738	0.793
Heterozygotes proportion	0.074	0	0.04	0.241	0.24	0.192	0.048	0.048	0.11
DRB-G-3									
Frequencies A	0.407	0.079	0.222	0.194	0.25	0.094	0.364	0.115	0.216
Frequencies G	0.593	0.921	0.778	0.806	0.75	0.906	0.636	0.885	0.784
Heterozygotes proportion	0.519	0.158	0.296	0.056	0.346	0.188	0.636	0.231	0.304
FABP4_Ch1_SNP1									
Frequencies A	0.968	0.983	1	1	1	0.984	1	0.883	0.977
Frequencies G	0.032	0.017	0	0	0	0.016	0	0.117	0.023
Heterozygotes proportion	0.065	0.033	0	0	0	0.032	0	0.167	0.037
FN1									
Frequencies C	0.286	0.341	0.08	0.36	0.341	0.357	0.188	0.042	0.249
Frequencies T	0.714	0.659	0.92	0.64	0.659	0.643	0.813	0.958	0.751
Heterozygotes proportion	0.214	0.318	0.16	0.4	0.409	0.333	0.292	0.083	0.276
GDFSNP452R									
Frequencies G	0.032	0.242	0.194	0.113	0.339	0.048	0.317	0.25	0.192
Frequencies T	0.968	0.758	0.806	0.887	0.661	0.952	0.683	0.75	0.808
Heterozygotes proportion	0.065	0.161	0.194	0.161	0.355	0.097	0.433	0.3	0.221

Table 2 *Continued*

Loci	Populations								
	German alpine	Verata	Corsican	Greek goat	Grigia molisana	Baladie	Polish fawn imp. goat	Brava	Means
GHR-G-1a									
Frequencies C	0.645	0.638	0.37	0.517	0.5	0.897	0.481	0.444	0.561
Frequencies T	0.355	0.362	0.63	0.483	0.5	0.103	0.519	0.556	0.439
Heterozygotes proportion	0.452	0.586	0.391	0.433	0.5	0.138	0.444	0.519	0.433
IL2_5p									
Frequencies C	0.048	0	0.032	0.048	0	0.032	0.048	0	0.026
Frequencies T	0.952	1	0.968	0.952	1	0.968	0.952	1	0.974
Heterozygotes proportion	0.097	0	0.065	0.097	0	0.065	0.097	0	0.052
IL2_In2									
Frequencies G	0.806	0.855	0.9	0.871	0.903	0.586	0.417	0.733	0.759
Frequencies T	0.194	0.145	0.1	0.129	0.097	0.414	0.583	0.267	0.241
Heterozygotes proportion	0.387	0.29	0.2	0.258	0.129	0.207	0.5	0.533	0.313
IL4SNP119R									
Frequencies C	0.29	0.661	0.629	0.484	0.645	0.887	0.4	0.3	0.537
Frequencies T	0.71	0.339	0.371	0.516	0.355	0.113	0.6	0.7	0.463
Heterozygotes proportion	0.387	0.419	0.419	0.387	0.452	0.161	0.4	0.467	0.387
ITGB1-G-2									
Frequencies G	0.194	0.4	0.397	0.267	0.379	0.065	0.155	0.25	0.263
Frequencies T	0.806	0.6	0.603	0.733	0.621	0.935	0.845	0.75	0.737
Heterozygotes proportion	0.387	0.467	0.448	0.467	0.414	0.13	0.31	0.367	0.374
Lact-G-1									
Frequencies A	0.107	0.192	0.306	0.167	0.518	0.25	0.103	0.25	0.237
Frequencies G	0.893	0.808	0.694	0.833	0.482	0.75	0.897	0.75	0.763
Heterozygotes proportion	0.214	0.231	0.226	0.333	0.393	0.167	0.207	0.214	0.248
LIPE-G-1									
Frequencies A	0.786	0.556	0.661	0.423	0.534	0.674	0.776	0.648	0.632
Frequencies C	0.214	0.444	0.339	0.577	0.466	0.326	0.224	0.352	0.368
Heterozygotes proportion	0.357	0.519	0.355	0.462	0.517	0.13	0.448	0.481	0.409
mel-G-1									
Frequencies C	0.554	0.648	0.435	0.667	0.722	0.87	0.534	0.685	0.639
Frequencies T	0.446	0.352	0.565	0.333	0.278	0.13	0.466	0.315	0.361
Heterozygotes proportion	0.321	0.407	0.613	0.519	0.407	0.261	0.517	0.407	0.432
MSTNG-5									
Frequencies A	0.952	0.967	0.931	0.897	0.964	0.935	0.933	0.969	0.943
Frequencies G	0.048	0.033	0.069	0.103	0.036	0.065	0.067	0.031	0.057
Heterozygotes proportion	0.032	0.067	0.069	0.138	0.071	0.13	0.133	0.063	0.088
PRP_EX3									
Frequencies A	0.661	0.452	0.387	0.419	0.452	0.194	0.724	0.483	0.471
Frequencies G	0.339	0.548	0.613	0.581	0.548	0.806	0.276	0.517	0.529
Heterozygotes proportion	0.484	0.516	0.581	0.516	0.581	0.258	0.345	0.3	0.448
PRP_IN2									
Frequencies C	0.597	0.45	0.357	0.389	0.333	0.341	0.722	0.467	0.457
Frequencies T	0.403	0.55	0.643	0.611	0.667	0.659	0.278	0.533	0.543
Heterozygotes proportion	0.548	0.5	0.524	0.481	0.37	0.5	0.333	0.267	0.441
TL4SNP214R									
Frequencies C	0.258	0.613	0.452	0.484	0.306	0.742	0.35	0.867	0.509
Frequencies T	0.742	0.387	0.548	0.516	0.694	0.258	0.65	0.133	0.491
Heterozygotes proportion	0.452	0.323	0.581	0.387	0.613	0.516	0.367	0.2	0.43
U80SNP129F									
Frequencies A	0.645	0.855	0.871	0.887	0.839	0.661	0.85	0.867	0.809
Frequencies C	0.355	0.145	0.129	0.113	0.161	0.339	0.15	0.133	0.191
Heterozygotes proportion	0.387	0.29	0.258	0.226	0.258	0.097	0.3	0.2	0.252
All loci									
Mean heterozygotes proportion	0.296	0.304	0.287	0.292	0.324	0.206	0.335	0.263	
Heterozygotes proportion standard deviation	0.161	0.187	0.182	0.169	0.197	0.152	0.155	0.168	

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